MEASURE COLOR STABILITY OF RED CABBAGE USING DIFFERENT SOLVENTS

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ABSTRACT

Color is one of most important properties of foods and beverages and is a basis for their identification and acceptability. The use of natural colorants has generated considerable interest nowadays as an alternative to synthetic colorants. Consumer concern over the safety of synthetic food colorants has increased the demand for natural food colorants. Our objective of this research is to determine the color stability of red cabbage based on storage days by using different solvents. Color was determined using CIE system L*, a*, b*. The color stability of red cabbage is based on L*, a*, b* values. Red cabbage was extracted with different volume concentration of solvents used. The solvents used for this extraction are methanol and ethanol. Based on the results, we can conclude that the color of red cabbage is most stable when extracted with 40% methanol.

ABSTRAK

Warna adalah salah satu ciri penting pada makanan dan minuman dan adalah asas kepada pengenalan dan penerimaannya. Penggunaan bahan pewarna semula jadi semakin menggalakkan kebelakangan ini sebagai alternatif kepada bahan pewarna tiruan. Kebimbangan pengguna terhadap keselamatan bahan pewarna tiruan merupakan faktor kepada peningkatan permintaan terhadap bahan pewarna semula jadi. Kajian ini bertujuan untuk menentukan kestabilan warna kubis merah berdasarkan pada masa penyimpanan dengan menggunakan pelarut yang berbeza. Warna ditentukan dengan menggunakan sistem CIE 1976 (L*a*b*). Kestabilan warna kubis merah adalah berdasarkan pada nilai L*, a* dan b*. Kubis merah diekstrak dengan menggunakan pelarut yang berbeza kepakatan isipadunya. Pelarut yang digunakan dalam pengekstrakan ini adalah metanol dan etanol. Berdasarkan kepada keputusan eksperimen, kesimpulan yang boleh dibuat adalah warna kubis merah adalah paling stabil apabila diekstrak menggunakan 40% metanol.

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LIST OF ABBREVIATIONS

FDA	-Food and Drug Administration
SPE	-Solid phase
LLE	-Liquid-liquid
CCC	-Counter current chromatography
MPLC	-Medium pressure liquid chromatography
HPLC	-High performance liquid chromatography
PDA	-Photodiode array
CIE	-Commission Internationale de I' Eclairage

LIST OF SYMBOLS

%	-	Percent
°C	-	Degree Celcius
g	–	Gram
v/v	-	Volume per volume
mL	-	Milliliter
K	-	Kelvin
mm^{-}	-	Millimeter
L*	-	Lightness or darkness
a*	-	Redness or greenness
b*	-	Yellowness or blueness
C*	-	Chroma
h*	-	Hue

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

The red cabbage is a sort of cabbage, which known as Red Kraut or Blue Kraut after preparation. The color of its leaves is dark red or purple. However, the colour of its plant is depends on the pH value of the soil, due to a pigment called anthocyanin. The leaves grow more reddish on acidic soil while an alkaline soil will produce rather greenish-yellow colored cabbages. This proves the fact that the very same plant is known by different colors in various regions. (Chigurupati *et al*., 2002).

Red cabbage dye is a natural pigment used mainly as a food color. Red cabbage coloring is currently used, to color various beverages, candies, dry mixed concentrates, chewing gums, yoghurts, and sauce. Investigations have been carried out to find out if it is possible to use it as an indicator of changes in the pH value in pharmaceutical preparations. (Saiki *et al*., 2002). Unlike the majority of the anthocyanins manufactured from berry fruits, the colorant obtained from red cabbage can be used to color food articles over a wide pH range, not only acidic products but also neutral ones. It can therefore replace synthetic blue dyes. (Dyrby *et al*., 2001).

The stability of red cabbage color is dependent on temperature, pH as well as concentration. The dye is most stable at room temperature and pH 3. It is least stable at 50°C and pH 8. Natural colorants are highly demand and have attracted interest rather than chemical colorant because of their safety and potential nutritional and therapeutic effect. Nowadays, consumers are concerned about the foods and

beverages they consume and how it affects their health and the health of their children.

The role of anthocyanin as food coloring agent is becoming increasingly important. They are not only contribute to the most important attributes of food, both for aesthetic value and for quality judgement but they also tend to yield potential positive health effects. (Pearce *et al*., 2002). The interest in anthocyanins derives not only from their coloring effect but also from their beneficial properties, including antioxidising activity, improvement in the tightness of capillary blood vessels and prevention of thrombocyte aggregation, all of which reduce the risk of circulatory diseases.(Degenhardt *et al*., 2000). Their antioxidant activity is so significant to human health that cases such as the "French paradox" have come to the fore (Renaud and De Lorgeril, 1992). As evidence, french people ingest great amounts of lipids but do not suffer from hypercholesterolemia. This has shown that the anthocyanins inhibit the oxidation of lipid. (Narayan *et al*., 1999).

1.2 Problem Statements

Natural plant colorants are in high demand by the food industry to replace chemical colorants. As we know, chemical colorants are widely used in various food items like ice creams, fruit drinks, sweet meats and others. Chemical colorants are only giving an attractive look to the item and add nothing to the nutritional value of the food.

Nowadays, people are very careful on choosing the right food to avoid them from taking the food which can give some problems to their health. Natural colorants such as red cabbage dye is quite important nowadays mainly as food color. It has a class of compounds called anthocyanins attributes to this color. Although anthocyanins have a high potential for use as natural colorants due to their attractive colors and innocuousness (Giusti and Wrolstad, 2003; Pazmino-Duran *et al*., 2001), they do present stability problems. The color and stability of anthocyanin pigments

are dependent on several factors, including structure, concentration, pH, temperature, light, presence of copigments, metallic ions, enzymes.

Thus this research is important to know the best condition at which the red cabbage is stable based on its storage days by using different concentration of solvents.

1.3 Objectives

The main objective of this research is to determine the color stability of red cabbage based on storage days by using different solvents and to determine the chemical constituents by using CIE lab method.

1.4 Scope of study

The scope of study for this research is about characterizing color using CIE method. The other scopes are components that contribute to the color of red cabbages, effect of the concentration difference in solvents for extraction and the differences in color properties based on the storage days. The results from parameters being tested remarked the best performance color stability can achieve at any given concentration of each solvent used.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Red cabbage (Brassica oleracea L. var. capitata f. rubra) is a native vegetable of South-western Europe and Mediterranean region. Nowadays, we can find this cabbage not only at this two region but all over the world. Red cabbage belongs to the family of Brassicaceae. Red cabbage is a promising source of anthocyanins for coloration of foods since its anthocyanins are unique in being coloured over a very broad pH-range compared to anthocyanins from, e.g. grape skin, black currant and elderberry, which only possess a reasonable degree of colour at pH<4 (Brouillard *et al*., 1987; Mazza *et al*., 1987). The colours of anthocyanins from red cabbage vary from red at low pH to blue and green at high pH (Mazza & Miniati, 1993). The anthocyanin composition of red cabbage is very complex, due to glucosylation of the anthocyanidin (cyanidin) with two different sugars and acylation with several aromatic acids. The analysis of red cabbage anthocyanins is a difficult task, due to the lack of pure and structurally defined commercially available standards of acylated cyanidins, the large concentration range of its anthocyanins, and also the complexity of red cabbage chromatographic profile due to the big number of anthocyanins contained (Charron *et al*., 2007; Dyrby *et al*., 2001; Wu & Prior, 2005; Wu *et al*., 2006).

2.2 Red Cabbage

Red cabbage can be defined as a mature cabbage with a strong, peppery flavor and tough leaves. The pH value of the soil gives the difference of its color. Its plant turn blue or purple after preparation. Red cabbage is also known as red kraut or blue kraut. It is commonly used for coleslaw and salads. Nowadays, red cabbage is popularly used as an alternative to green cabbage to add color and presentation to salads and cooked dishes.

Red cabbage dye is a natural pigment used mainly as a food color. The red color of cabbage can turn many different colors when prepared incorrectly. These color changes are due to a pigment called anthocyanin. (Gayser *et al*., 2002). Red cabbage should be cooked with vinegar in order to avoid this color changes. Red cabbage takes about 70-75 days to harvest, similar to the time frame of a green cabbage, but faster than a savoy cabbage. (Matthew, 2003). Red cabbage is generally smaller and denser than green cabbage. Red cabbage should be harvested in well-fertilized soil in the late winter or early spring. The cabbage is often planted nearly a month prior to the last frost of the year. (Matthew, 2003).

2.3 Anthocyanins

Anthocyanins (in Greek *anthos* means flower, and *kyanos* means blue) are water soluble vacuolar pigments that may appear red, purple or blue according to pH. Many anthocyanins are red at acidic conditions and turn blue at less acidic conditions. Anthocyanins occur in all higher plants, mostly in flowers and fruits but also in leaves, stems, and roots. In these parts they are found predominantly in outer cell layers. (Lauro *et al*., 2000). They have long been the subject of investigation by botanists and plant physiologists because of their roles as pollination attractants and phytoprotective agents. They have also been very useful in taxonomic studies. (Wrolstad, *et* al., 2001). The colour of anthocyanins depends not only on the structure, but also on the acidity of the fruit. Chemically anthocyanins are subdivided

into the sugar-free anthocyanidine aglycons and the anthocyanin glycosides. They are used as food additive. (Hendry *et al*., 1996).

2.3.1 Structure

Anthocyanins are glycosides of one of several forms of anthocyanidins (aglycone), which differ from one another in the position of substitution of hydroxyl and methoxy-groups in the β ring of the flavylium cation. As shown in the figure below, the anthocyanins are based on a single basic core structure.

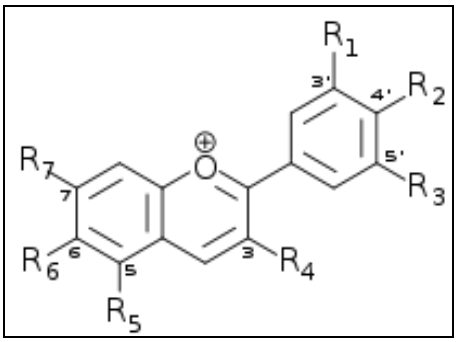

Figure 2.1 The flavylium ion. (Francis *et al*., 2000)

There are seven different side groups on the flavylium ion which can be a hydrogen atom, a hydroxide or a methoxy-group. The most frequent combination of side groups and their names are shown in Table 2.1.

Table 2.1: Main groups of anthocyanidins (Hendry *et al*., 1996)

Anthocyanidin	**R_1**	**R_2**	**R_3**	**R_4**	**R_5**	**R_6**	**R_7**	**main colour**	**E-number**
Apigeninidin	-H	-OH	-H	-H	-OH	-H	-OH	orange	
Aurantinidin	-H	-OH	-H	-OH	-OH	-OH	-OH	orange	
Capensinidin	$-OCH_3$	-OH	$-OCH_3$	-OH	$-OCH_3$	-H	-OH	bluish-red	
Cyanidin	-OH	-OH	-H	-OH	-OH	-H	-OH	Magenta	E163a
Delphinidin	-OH	-OH	-OH	-OH	-OH	-H	-OH	purple, blue	E163b
Europinidin	$-OCH_3$	-OH	-OH	-OH	$-OCH_3$	-H	-OH	bluish red	
Hirsutidin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	$-OCH_3$	bluish-red	
Luteolinidin	-OH	-OH	-H	-H	-OH	-H	-OH	orange	
Pelargonidin	-H	-OH	-H	-OH	-OH	-H	-OH	orange, salmon	E163d
Malvidin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	-OH	purple	E163c
Peonidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	-OH	Magenta	E163e
Petunidin	-OH	-OH	$-OCH_3$	-OH	-OH	-H	-OH	purple	E163f
Pulchellidin	-OH	-OH	-OH	-OH	$-OCH_3$	-H	-OH	bluish-red	
Rosinidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	$-OCH_3$	red	
Triacetidin	-OH	-OH	-OH	-H	-OH	-H	-OH	red	

In plant products, anthocyanins occur in the form of mono-, di- and triglycosides. Anthocyanin glycoside residues are, in turn, frequently acylated with phenolic acids. Both glycosidation and acylation of glycoside residues increase anthocyanin stability (Bridle and Timberlake, 1997; Brouillard, 1982; Giusti and Wrolstad, 2003). The principal aglycone of red cabbage is cyanidin, which occurs as cyanidin 3-sophoroside-5-glucoside and cyanidin 3,5-diglucoside, acylated with sinapic, ferulic, malonic and *p*-coumaric acids (Hrazdina *et al*., 1977; Sapers *et al*., 1981; Tanchev and Timberlake, 1969). This aglycone core can exist as a positively charged oxonium ion and termed as a flavylium cation in acidic solution. The

flavylium cation can exist in equilibrium with a colorless pseudo-base form in basic pH.

The major anthocyanins of red cabbage are based on a core of cyanidin-3-*O*-diglucoside-5-*O*-glucoside which can be non-acylated, mono-acylated or di-acylated with *p*-coumaric, caffeic, ferulic and sinapic acids (Tanchev and Timberlake, 1969; Giusti *et al*; Wu and Prior, 2005). Anthocyanins exist in equilibrium of four molecular species; the coloured basic flavylium cation and three secondary structures; the quinoidal bases, the carbinol pseudobase and the chalcone pseudobase forms. At pH 2 or below, the flavylium cation form predominates but as the pH is raised towards 7 the colourless chalcone pseudobase begins to dominate. The unusual pH stability of the colour of red cabbage anthocyanins is thought to be due to the presence of these acyl groups which "hinder the hydrolysis of the red flavylium cationic form to the colourless carbinol base, allowing preferential formation of the blue quinoidal bases" (Bridle and Timberlake, 1997). Glycosylation at positions 3 and 5 shifts the colour towards the blue and the stability of colour may also be influenced by intramolecular co-pigmentation (Maulien-Aubert *et al*., 2001).

2.3.2 Functions

There are many functions of anthocyanins. Anthocyanins, as natural colorants, are widely used in the food industry as an alternative to synthetic colorants. The interests in and motives for extended use of these colorants are influenced by their potential beneficial health effects. (Clifford, 2000). Another favourable aspect of anthocyanins is that they contribute greatly to the antioxidant properties of certain foods. (Einbond *et al*., 2004).

There is also an increasing interest in anthocyanins because of their potential health-promoting properties and, above all, for their protection against free radicals (Rossetto *et al*., 2002; Saint-Cricq de Gaulejac *et al*., 1999). Anthocyanins have a range of biological activities that may produce health benefits for examples range from inhibition of DNA damage in cancer cells in vitro (Hou, 2003), inhibition of

digestive enzymes (McDougall and Stewart, 2006) induction of insulin production in isolated pancreatic cells (Jayaprakasam *et al*., 2005), reduction in inflammatory responses (Tall *et al*., 2004) to protection against age-related decline in brain function (Lau *et al*., 2006).

The most significant function of anthocyanins is their ability to impart color to the plants or plant products in which they occur. They play a definite role in the attraction of animals for pollination and seed dispersal, and hence they are of considerable value in the co-evolution of these plant-animal interactions.

2.3.3 Stability of anthocyanins

Anthocyanins are natural colorants which are widely used nowadays in food industry as food coloring agent due to their extensive range of colors, innocuous and beneficial health effects. The applications of anthocyanins in food, pharmaceutical and cosmetic industries has been limited due to their relative instability and low extraction percentages.

The isolated anthocyanins are highly instable and very susceptible to degradation (Giusti & Wrolstad, 2003). Their stability is affected by several factors such as pH, storage temperature, chemical structure, concentration, light, oxygen, solvents, the presence of enzymes, flavonoids, proteins and metallic ions (Rein, 2005). The anthocyanins chemical stabilisation is the main focus of recent studies due to their abundant and potential applications, their beneficial effects and their use as alternative to artificial colorants (Rein, 2005).

Based on molecular structure, some anthocyanins are more stable than others. For example, malvidin glycosides, the main anthocyanins in grapes, are among the most colour-stable, due to dimethoxylation of the molecule (Bridle and Timberlake, 1997). Also, acylation with hydroxylated aromatic organic acids confers higher stability, with few exceptions (Bassa and Francis, 1987; Francis, 1989).

Stability of anthocyanins can also increase with inter molecular copigmentation (Francis, 1989; Malien-Aubert *et al*., 2001). Aqueous fruit, vegetable, and grain extracts, with high anthocyanin content, contain mixtures of different compounds that may serve as copigments for intermolecular association with anthocyanins. However, not all compounds enhance copigmentation; for example, sugars and their degradation products tend to accelerate the degradation of anthocyanins. The rate of anthocyanin degradation is associated with the rate at which the sugar is degraded to furfural-type compounds derived from the Maillard reaction (Duhard *et al*., 1997).

2.3.4 Distribution and content of anthocyanins in fruits and vegetables

Anthocyanins are water-soluble and vacuolar pigments found in most species in the plant kingdom (Harborne 1998). They are accumulated in fruit plants such as blackberry, red and black raspberries, currants and vegetables such as: red onion, radish, red cabbage, fennel, red-skinned potato and purple sweet potato. Total anthocyanins content varies considerably among different plants affected by genes, light, temperature, and agronomic factors. The level of anthocyanins in fruits is much higher than in vegetables.Anthocyanins can be found in all parts of the plants. Although they are accumulated mostly in flowers and fruits, but are also present in leaves, stems and storage organs (Brouillard, 1982; Delgado-Vargas and Paredes-López, 2003).

2.4 Extraction Methods

The solvent extraction has been the most common method for extraction of diverse compounds found in fruits and vegetables. Anthocyanins are polar molecules, thus the most common solvent used in the extractions are aqueous mixtures of ethanol, methanol or acetone (Kahkonen *et al*., 2001). The choice extracting medium used to extract red cabbage is very important. It should maximize

pigment recovery with a minimal amount of adjuncts and minimal degradation or alteration of the natural state.

Among the most common methods are those which use acidified methanol or ethanol as extractants (Amr and Al-Tamimi, 2007; Awika *et al*., 2005; Cacace and Mazza, 2003; Donner *et al*., 1997; Fossen and Andersen, 2003; Phippen and Simon, 1998). From these methods, the extraction with methanol is the most efficient (Kapasakalidis *et al*., 2006). In fact, the anthocyanin extraction with methanol from grape pulp is 20% more effective than with ethanol and 73% more effective than only water (Metivier *et al*., 1980). However, extraction with ethanol is used in industry due to the methanol toxicity.

In acidified solvent extraction, precaution steps should be taken to avoid strong acid media because the acylated anthocyanin might be degraded (hydrolysis reaction) and in the case of 3-monoside anthocyanins the glycoside bonds could be destroyed (Kapasakalidis *et al*., 2006).

To obtain anthocyanins closer to their natural state, a number of researchers have performed the initial extraction using neutral solvents such as 60% methanol, *n*-butanol, cold acetone, acetone/methanol/water mixtures, or simply water (Jackman *et al*., 1987). Others have isolated anthocyanin pigments with mixtures of methanol/acetic acid/water (10:1:9, v/v/v) (Takeda *et al*., 1986; Davies and Mazza, 1992), ethanol/acetic acid/water (12:1:24, v/v/v) (Toki *et al*.,1991) and (10:1:9, v/v/v) (Hosokawa *et al*., 1995) and methanol/formic acid/water (50:5:45, v/v/v) (Donner *et al*.,1997).

2.4.1 Extraction with methanol

This is the classical method of extracting anthocyanins from plant materials. Methanol is the most commonly used solvent for anthocyanin extraction because its low boiling point allows for rapid concentration of the extracted material. However, the resultant extract contains low-polarity contaminants and further purification may

be necessary. Methanol extraction is a rapid, easy, and efficient method for anthocyanin extraction (Rodriguez-Saona and Wrolstad, 2001**)**. However, methanol is not preferred for food use to avoid the toxicity of methanolic solutions.

2.4.2 Extraction with ethanol

It may be noted that the 100% alcohol (ethanol alone) is not preferable as an extracting solvent because the presence of a little water is required for the extraction of hydrophilic anthocyanins. The anthocyanin content in extract has increased with an increase in percentage of alcohol in water. Water: ethanol mixture of 80:20 (v/v) is commonly used as a solvent in the food industry, and it is as good as methanol (Lapornik *et al*., 2005). Ethanol is a versatile solvent. It can miscible with water and with many organic solvents such as acetone, benzene, acetic acid and toluene. Ethanol-water mixtures have less volume than the sum of their individual components at the given fractions. The mixture of ethanol and water is exothermic **(**Sowerby and Crittenden, 1988**).** Mixtures of ethanol and water form an azeotrope at about 89 mole-% ethanol and 11 mole-% water at normal pressure and T = 351 K.

2.4.3 Extraction with acidified water

The extraction of anthocyanins was more in case of acidified water when compared to the pure water. This can be mainly attributed to the presence of hydrochloric acid which stabilizes the pigments and lowers a pH to a level where the absorbance of the anthocyanins is at their maximum. The anthocyanin content in extract increased with an increase in percentage of acid in water (Patil *et al*., 2007). The use of acidic solvents, contribute to denature the membranes of cell tissue and simultaneously dissolve pigments.

2.4.4 Extraction with alcohol in acidified water

In order to extract more anthocyanin, it was better to use the alcohol in acidified water. In aqueous extractions, the most used and efficient acids are acetic, citric, tartaric and hydrochloric. The acid tends to stabilize anthocyanins, but it may also change the native form of the pigment in the tissue by breaking associations with metals, co-pigments, or other factors. The anthocyanin content in extract increased with an increase in percentage of alcohol in acidified water. However, the presence of alcohol in the natural color extract may limit its application as food colorant. (Madhusudhan *et al*., 2007).

2.4.5 Purification Methods

The extraction methods proposed up to now are not selective for anthocyanins, since they are able to co-extract a great number of other compounds, such as sugars or organic acids (Coutinho *et al*., 2004). Thus, new purification techniques are recommended in order to isolate the anthocyanins of interest.

In this sense, it has been proposed a wide variety of techniques, from extractions in solid phase(SPE) and liquid-liquid (LLE) (Donner *et al*., 1997; Fossen and Andersen, 2003; Romani *et al*., 1999) up to the use of sophisticated chromatographic techniques like countercurrent chromatography (CCC) (Schwarz *et al*, 2003), medium pressure liquid chromatography (MPLC) (Vivar-Qintana *et al*., 2003) and the high performance liquid chromatography(HPLC) (Alcalde-Eon *et al*., 2004). The CCC and MPLC are used as purification methods with subsequent analysis by HPLC for structural elucidation, with the advantage of minimizing the separation time and mobile phase solvents (Mikanagi *et al*., 2000). The most common method used for anthocyanins separation is HPLC with UV-Vis or photodiode array (PDA) detectors (Missang *et al*., 2003; Mikanagi *et al*., 2000).

2.5 Colors

The color of a food is the first quality factor that the consumer appreciates and has a remarkable influence on its acceptance. Color is also an indicator of the natural transformation of a fresh food (ripeness) or of changes that occur during its storage or processing. Color derives from the spectrum of light (distribution of light energy versus wavelength) interacting in our eye with the spectral sensitivities of the light receptors. Physical specifications of color and color categories are based on their physical properties such as light absorption, reflection or emission spectra. The colors of visible light spectrum follow this range of values:

- Violet blue = $380 < \lambda < 480$ mm
- Green = $480 < \lambda < 560$ mm
- Yellow = $560 < \lambda < 590$ mm
- Orange = $590 < \lambda < 630$ mm
- Red = $630 < \lambda < 750$ mm

Visible light where its wavelength is between 380-750 mm, are very important to color appreciation (Delgado-Vargas and Paredes-Lopez, 2002).

Color is an important sensory property in determining product quality, therefore minimizing the pigment losses during processing is of primary concern to the processor (Markakis, 1982; Bridle and Timberlake, 1997). The visual color, which is an indicator of pigment concentration, can be measured instantaneously using tristimulus colorimeters for on-line quality control (Rocha *et al*., 1993).

2.5.1 Purpose of food coloring

Certain colors normally are associated with certain flavors and the perceived flavor is influenced by the color of food in anything from candy to wine. (Delwiche, 2004). So it caused food manufacturers to add dyes to their products. Sometimes the